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(54) Title: METHOD OF SCREENING AND CLASSIFYING COMPOUNDS

(57) Abstract

The invention is directed to a method for classifying bioactive compounds. The method comprises evaluating the effect of an unclassified compound on a series of nematodes selected from the group consisting of wild-type, stable mutant nematodes, or both; and, characterizing the compound as being in the same class as other compounds which give a similar response profile to said series of nematodes or in a class different from those classes of compounds already recognized by response profile.

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TITLE OF THE INVENTION

METHOD OF SCREENING AND CLASSIFYING COMPOUNDS

This application is a continuation-in-part application of U.S. Patent Application Serial No. 07/305,835, filed February 3, 1989.

This invention was made with government support, and the government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to a method for screening and classifying compounds for bioactivity by use of wild-type and genetically altered nematode mutants, particularly stable mutant strains of the nematode Caenorhabditis elegans.

These methods are useful for the classification of synthetic compounds and natural products into pharmacological and biochemical classes defined by a mechanism or mode of action.

These methods are also useful for the identification of desired properties in synthetic compounds and natural products

which have application as human or veterinary pharmaceuticals, research agents, anthelmintics, nematocides, pesticides, insecticides, fungicides, parasiticides, endectosides and the like.

In addition, these methods are useful as assays to monitor the purification of a bioactive agent or bioactive combination of agents.

Description of the Background Information

A. Screening Methods

A large number of methods have been developed to screen compounds for the identification and characterization of bioactivity. These methods vary significantly both in their ease of performance and expense and in the amount of information which they yield regarding the mode of action of the compound being tested.

a) At one extreme are methods which screen for the bioactivity of a compound directly on the disease or infection which it is desirable to control; for example, bioactivity may be evaluated directly in a human burdened with the disease or medical condition, or, for example, bioactivity may be evaluated directly on an agricultural crop infected with a pest. Such methods are often difficult and expensive; they are clearly of limited use for the identification of new or improved human therapeutics. Furthermore, direct screens, by their very nature, provide little or no information regarding the mode of action of the compounds being tested. In addition, a direct screen will fail to identify potential lead compounds for the development of active compounds when the activity of the lead compound is masked by metabolism or by lack of access to the target tissue.

b) A common alternative to direct screening in humans involves testing compounds in animal models. The identification of many human therapeutics has emerged from screens which utilize special strains of mammals, and especially rodents, as models for a particular human disease. Although such methods are valuable, they are limited by the availability and applicability of the appropriate animal models. Furthermore, the difficulty and expense of these systems remains relatively high and, as with direct screens, they provide no information on the mode of action of active compounds and may fail to identify interesting lead compounds. In addition, studies which utilize higher vertebrates are highly regulated and limited by growing concerns for laboratory animal welfare.

c) In vitro screening methods which are target-specific have been used to identify compounds with a desired bioactivity. These methods are generally of two types: physiological screens in which intact tissues or isolated cells respond specifically and characteristically to compounds of the desired mode of action; and biochemical screens in which the ability of compounds to alter a characteristic enzymatic activity or ligand binding is measured.

These methods, by definition, yield information on the mode of action of the compound being examined. However, because in vitro screening is mode of action-specific, it is necessary to perform a completely different analysis for every pharmacological or biochemical activity being tested. This makes it difficult and expensive to methodically screen large numbers of compounds for a wide variety of pharmacological or biochemical activities.

In vitro physiological screens are amenable to the identification of interesting lead compounds. However, physiological screens are usually technically difficult and

many are limited by the availability of the animal tissues or cells.

In vitro biochemical screens can be rapid, and relatively inexpensive. Nonetheless, these screens can also be technically sophisticated as they require uniform, purified preparations of the biochemical target, often a labile enzyme or receptor. In addition, these assays are subject to a variety of artifacts resulting from the removal of the target enzyme or receptor from its natural environment. Also, biochemical screens may not differentiate well between an agonist and an antagonist.

d) Finally, at the extreme opposite to direct screening, methods based on computer modeling can be used to identify active compounds by predicting their three-dimensional interactions with their target macromolecule such as a protein or nucleic acid. See U.S. Patent No. 4,704,692. Currently, the value of this technique is extremely limited since it is based on knowing the three-dimensional structure of the target macromolecule and to date this information is available for only a very few systems.

Thus, there remains a need for compound screening methods which i) rapidly and economically screen large numbers of compounds, ii) for a variety of bioactivities, iii) in a non-vertebrate, in vivo animal model, and iv) provide information as to the compound's mode of action.

B. The Nematode Caenorhabditis elegans

The free-living soil nematode Caenorhabditis elegans is a simple invertebrate animal which is small (adults of both sexes, hermaphrodites and males, are approximately 1 mm long) and easily cultured in the laboratory. Methods for growing C.

elegans are well-known to those skilled in the art. Brenner, S., Genetics 77:71-94 (1974); "The Nematode Caenorhabditis elegans," W.B. Wood, ed., 1988, Cold Spring Harbor Laboratory. For example, C. elegans can be grown either on agar surfaces seeded with Escherichia coli bacteria as a food source or in liquid cultures containing E. coli. Under optimal conditions eggs develop into egg-laying adults in less than 3 days; unmated hermaphrodites produce approximately 300 progeny. Thus it is easy to produce large numbers of animals and assays utilizing nematodes can be performed rapidly and in small volumes. These advantages have been noted by others who have used C. elegans as a test organism for anthelmintic and nematocide evaluation. Ohba, K. et al., J. Pestic. Sci 9:91-96 (1984); Vanfleteren, J.R. et al., Nematologica 18:325 (1972); Platzer, E.G. et al., J. Nematol. 9:280 (1977); Simpkin, K.G. et al., J. Chem. Tech. Biotechnol. 31:66 (1981); Spence, A.M. et al., Can. J. Zool. 60:2616 (1982); Popham, J.D. et al., Environ. Res. 20: 183 (1979).

Over the last 15 years, the biology of C. elegans has been the subject of an intense scientific research effort. As a result, this organism is now genetically and biologically the best understood metazoan species. Wood, supra; Kenyon, C., Science 240:1448-1453 (1988). Methods for the generation, isolation, and analysis of single gene mutations have been developed and are facilitated by the rapid growth and ease of culture noted above as well as by ability of hermaphrodites to reproduce by self-fertilization.

Many mutant nematode strains have been described which display specific, characteristic responses to different classes of chemical agents. For example, Brenner, S., Genetics 77: 71-94 (1974), among others (review Rand, J.B. et al., Psychopharm. Bull. 21:623-630 (1985)), discloses a series of mutants of C. elegans which are resistant to the cholinesterase inhibitors aldicarb, lannate or trichlorfon. Rand et

al., supra, note that mutants resistant to one of these compounds are resistant to all three.

Brenner, supra, as well as Lewis, J.A. et al., Neurosci. 5:967-989 (1980), and Lewis, J.A. et al., Genetics 95:905-928 (1980), disclose the construction of a series of mutants resistant to the anthelmintic levamisole. The authors identify three classes of mutants based upon phenotypic analysis. The authors state that the most resistant class of mutants might lack one class of pharmacologically functional acetylcholine receptors.

Tabuse, Y. et al., Carcinogenesis 4:783-786 (1983), discloses the construction of a set of nematode mutants which are resistant to the mammalian tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA).

In addition to mutants directed against membrane localized events, many mutants with intracellular targets have been described. For example, Sanford, T. et al., J. Biol. Chem. 258:12804-12809 (1983), discloses the isolation of mutants which are resistant to the RNA polymerase II inhibitor, α-amanitin. The authors discuss the use of these mutants to identify structural genes encoding subunits of RNA polymerase or its effectors.

Nematodes have been shown to respond to a variety of bioactive compounds in the same chemical form as that to which the higher vertebrates respond. For example, Morgan, P.G. et al., Anesthesiology 62:738-744 (1985), discloses the identification of a series of mutants which respond characteristically to volatile anesthetics. The mutants are characterized through their phenotypic expression in response to induction of anesthesia.

Trent, C. et al., Genetics 104:619-647 (1983), discloses the construction of mutants that are defective in egg-laying. The authors define four distinct categories of mutants based

on their responses to the pharmacological agents serotonin and imipramine.

To date, over 700 genes have been identified by mutations. Wood, supra, pp. 502-559. Stable strains carrying these mutations are easily maintained either on agar plates or, for long term storage, frozen in liquid nitrogen.

Thus, the nematode C. elegans provides a versatile, non-vertebrate, in vivo animal model of the higher eukaryotes, which is economical to maintain, technically simple to utilize, amenable to genetic manipulation, and inherently responsive to bioactive agents which modulate pharmacological and biochemical activities in the higher vertebrates and, especially, in man.

SUMMARY OF THE INVENTION

The invention provides a quick, reliable and accurate method of objectively classifying compounds, including human pharmaceuticals.

The invention further provides a method of identifying potentially useful medicinal, therapeutical, pharmacological, biochemical or biological properties in compounds.

The invention further provides a method of identifying and classifying the mechanism of action of a bioactive compound.

The invention further provides an assay to monitor the isolation or purification of a bioactive compound or bioactive mixture of compounds from a crude preparation.

In detail, the invention provides a method of classifying and identifying a compound's bioactivity and comprises comparing the activity profile which results from exposing a library of nematode mutants to that compound with the activity profile which results from exposing an identical library of

mutants to compounds of known medicinal, therapeutical, pharmacological, biochemical, morphological or biological effect, including cell death.

Additionally, one may identify compounds which affect biological processes such as cell death by exposing compounds of unknown biological activity to mutants wherein the effect of the compound may be demonstrated morphologically or through a change in cell death dependent behavior.

Consequently, the invention embraces any method which uses nematodes, including stable nematode mutants, for classification or identification of a compound by exposing said nematodes or nematode mutants to the compound and observing a response in the nematodes or nematode mutants and predicting the utility or presence of a compound based on a similar or dissimilar response in the nematodes or nematode mutants to other already classified groups of compounds. That response may be a behavioral (physical) response such as paralysis or a change in the locomotory, egg-laying, or taxis behavior of the nematode; or, the response may be a molecular response such as a change in the level of metabolite, protein, receptor, enzyme, or gene expression; or, the response may be a morphological and/or behavioral response such as the induction or prevention of cell death.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention is based on the inventor's realization that wild-type and mutant nematodes which have been discovered to respond in a defined, characteristic manner to a known compound or class of compounds, will respond in that same characteristic manner if they are exposed to an agent which acts through the same mode of action as that of said known compound or class of compounds. The inventor concluded it is possible to identify the presence of bioactivity in a

compound, and further, the mode of action and hence utility of that bioactivity, by merely examining the response of wild-type and mutant nematodes to the presence of a compound.

The present invention offers an objective method of identifying, sorting and classifying bioactive synthetic compounds or natural products based upon the ability of that bioactive compound or natural product to induce a predictable, qualitative response in wild-type nematodes, stable nematode mutants, or in both wild-type nematodes and stable nematode mutants.

DEFINITIONS

In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

Activity Profile. The results of a screen of a compound against a library of nematodes. The library may include both wild-type and mutant strains of nematodes.

Lead Compound. A lead compound is a compound which has been identified as having an inherent bioactivity and whose structure is used to create derivatives with enhanced bioactivity or application.

Receptor. The term "receptor" is intended to refer generally to a functional macromolecule or complex of macromolecules with which certain groups of cellular messengers, such as hormones and neurotransmitters, must first interact before the biochemical and physiological changes that are characteristic of their response are initiated. Therefore, as used herein, the term "receptor" is used operationally to denote any cellular macromolecule (or complex of macromolecules) to which a chemical or macromolecular entity specifically binds to initiate its effects.

Agonist. The term "agonist" is intended to refer generally to a chemical agent which mimics at least some of the effects of an endogenous physiological compound by interaction with the appropriate physiological receptor for that compound.

Antagonist. The term "antagonist" is intended to refer generally to a chemical compound which is able to bind to an endogenous physiological receptor, but which has no intrinsic regulatory activity at that receptor. As recognized by those skilled in the art, the binding of an antagonist results in an interference with the effect of the natural ligand or with an agonist. As used herein, compounds that are themselves devoid of intrinsic pharmacological activity but cause effects by inhibition of the action of a specific agonist, by competition for agonist binding sites, are designated as antagonists.

Agent. The term "agent," for example an antihistamine agent, is intended to refer generically to any compound which interacts with the appropriate receptor, macromolecule or response. In the above example, an antihistamine agent would be any compound which stimulates the antihistamine response, or the response of the antihistamine pathway. In another example, an anti-tubulin agent would alter tubulin expression, synthesis or turnover.

Nematode. The term "nematode" is intended to refer generally to the class Nematoda or Nematoidea and comprises those animals of a slender cylindrical or thread-like form commonly called worms.

Mutant. The term "mutant," as in "nematode mutant" or "mutant nematode," is intended to refer generally to a nematode which contains a stably altered genotype. The altered genotype results from a mutation not generally found in the genome of the wild-type nematode.

Library. A "library" of nematodes is a collection of

different nematodes and may include both wild-type and mutant nematodes.

Class. The term "class" is intended to refer generally to any common property shared within a group of compounds. That property may be a chemical one inherent to the compounds or it may be a bioactivity shared by a group of compounds.

Response. The term "response" is intended to refer to a change in any parameter which can be used to measure and describe the effect of a compound on a nematode. The response may be a physical one such as a change in the growth rate, locomotory behavior, paralysis, fecundity, or a change in the feeding habits of the nematodes; or, it may be a molecular one such as a change in a level of a metabolite, protein, receptor, enzyme, or genomic expression. Detection of the response is performed either by direct visual observation of the nematodes or by secondary analysis of the nematode-produced constituent.

Respond Characteristically. A nematode mutant which "responds characteristically" or has a "characteristic response" to the presence of a compound, responds to that compound in a manner indicative of a defined bioactivity. For example, a mutant characterized as being growth resistant to one class of nematocides "responds characteristically" by growing and replicating at concentrations of the nematocide that kill, or prevent the growth and replication of, other nematodes; the "characteristic response" of that mutant is growth in concentrations of the nematocide that kill or prevent the growth and replication of other nematodes.

Compound. The term "compound" is intended to refer to a chemical entity, whether in the solid, liquid, or gaseous phase. The term 'compound' should be read to include synthetic compounds, natural products and macromolecular entities such as polypeptides, polynucleotides, or lipids and also small entities such as neurotransmitters, ligands, hormones or

elemental compounds. The term "compound" is meant to refer to that compound whether it is in a crude mixture or purified and isolated.

Bioactive Compound. The term "bioactive compound" is intended to refer to any compound which induces a measurable response in an animal.

Anti-nematode Compound. The term "anti-nematode compound" is intended to include both anthelmintics and nematocides, that is, any compound or combination of compounds capable of killing, destroying, inhibiting the growth or reproduction of, or otherwise interfering in the normal physiology or metabolism of a nematode in a manner not conducive to the nematode's survival or reproduction.

Representative Compound. The term "representative compound" is intended to refer to a compound which possesses a bioactivity specific for a given class of compounds such that expression of this specific bioactivity may be used as a marker of that class.

Resistant. The term "resistant" refers to the ability of a mutant nematode to respond or survive in the presence of a concentration of a compound which paralyzes, kills or otherwise adversely affects the behavior, growth or reproductive system of the wild-type animal.

Resistance-selected. The term "resistance-selected" or "selected for resistance to" refers to the selection of a mutant nematode by its ability to respond characteristically to a concentration of a compound which inhibits or represses the ability of the wild-type nematode to respond.

Cross-resistance. The term "cross-resistance" or "cross-resistant" refers to the ability of a mutant to be resistant to more than a single compound or class of compound.

Cell Death. The term "cell death" is meant the process through which cells die, and which is generally followed by cell phagocytosis and degradation of cell remnants. As

referred to for the purposes of this invention, cell death is meant to exclude those processes wherein cells die as an immediate result of acute physical injury.

There are two types of cell death, apoptosis and necrosis. Necrosis occurs when cells are exposed to noxious stimuli such as hypoxia, ischemia, or toxins, or involved in complement-mediated lysis. Necrosis is sometimes characterized morphologically as swelling necrosis as the necrotic cell first swells and then the plasma membrane ruptures. Although changes in the cell's organelles are seen microscopically, the chromatin remains largely unchanged throughout the necrosis.

Apoptotic cell death results in a different and distinct pattern of structural changes. Apoptosis (sometimes called programmed cell death or shrinkage necrosis) is characterized by cell shrinking. The cell first rounds up, and then separates from its neighboring cells. Cell organelles remain relatively normal as the cytoplasm shrinks. However, striking changes occur in the nucleus and the chromatin condenses on the nuclear membrane. The final stage in apoptosis is the selective phagocytosis of the apoptotic cell. The apoptotic cell is easily discernable upon microscopic examination by the presence of apoptotic bodies which contain fragmented DNA. Thus, it is possible to morphologically distinguish whether a cell has died through apoptosis or necrosis by microscopic techniques.

The chemical, pharmacological or bioactivity class to which a compound or natural product may be assigned using the method of the invention is limited only by the ability of that chemical, pharmacological or bioactivity class to induce a detectable change in nematode physiology, activity, metabolism or biochemistry. The class may be restrictive or broad. An example of a restrictive class is a class defined by the

activity profile of a specific compound or drug. An example of a broad class is a class defined by the activity profile of a pharmacological activity shared by a group of compounds. Accordingly, examples of classes of compounds within which a bioactive compound or natural product may be classified by the method of the invention include classes represented by biochemical and pharmacological activities and classes represented by specific drugs, therapeutical agents, biochemicals, enzyme inhibitors and activators, receptor agonists and antagonists, and other effectors which associate with cellular components.

Examples of the types of effectors which may be used in establishing the class of a compound include: anthelmintics, nematocides, insecticides, endectosides, parasiticides, and fungicides as well as veterinary or human pharmaceuticals including anaesthetics and antidepressants. Examples of types of compounds which may be used in establishing the class of a compound include: acetylcholine agonists and antagonists such as levamisole, morantel, and pyrantel; anticholinesterase inhibitors such as physostigmine, aldicarb, trichlorfon, and paroxon; GABA agonists and antagonists such as piperazine and muscimol; dopamine agonists and antagonists, anti-tubulin agents, serotonin agonists and antagonists such as 1-(2,5-dimethoxy-4-indophenyl)-2-aminopropane, quipazine, 1-(1-naphthyl)piperazine, and ketanserin; serotonin uptake blockers generally, and in particular subclasses of serotonin uptake blockers, for example, subclass A serotonin uptake blockers such as clomipramine and amitriptyline and subclass B serotonin uptake blockers such as fluoxetine and paroxetine as defined in Example 5.

The present invention envisions that one of ordinary skill may expose nematodes to the compound being tested either by direct injection or by external exposure with subsequent absorption, adsorption, binding or ingestion of the compound.

The present invention also envisions that the effect of a wide range of concentrations of the compound would be examined. In one embodiment, the compound being tested is injected directly into the nematode. In a preferred embodiment, the nematode is placed on an agar surface which contains the compound or into a liquid media or solution which contains the compound.

An extensive listing of wild type and mutant C. elegans strains which are useful in the methods of the invention is provided in "The Nematode Caenorhabditis elegans," W.B. Wood, ed., 1988, Cold Spring Harbor Laboratory. The strains listed herein, or comparable strains thereto, can be obtained from the Caenorhabditis Genetics Center at the University of Missouri, Columbia, Missouri.

In a preferred embodiment of the invention, a compound whose bioactivity is unknown is first tested against the wild-type nematode to identify bioactivity and then tested against a library of nematode mutants to classify the mechanism of action of that bioactivity. Compounds such as anthelmintics or nematocides reveal their bioactivity when presented to the wild-type nematode. Therefore in a first or primary screen for some classes, the compound being tested is evaluated for its ability to alter the growth, movement, metabolism, reproduction or other response in the wild-type nematode. In a secondary screen, compounds revealing bioactivity from the first screen are sorted and classified by the activity profile of stable nematode mutants which are exposed to said compound. The compound would be classified and the mechanism of action identified as a function of the class or classes of mutants which were resistant or otherwise responded to the compound in a characteristic manner.

For example, to classify the mode of action of a compound identified as having anti-nematode activity against wild-type nematodes, a library of different mutants which, in total, includes at least one mutant strain representing every

known class of anti-nematode compounds would be used. In this example, if none of the mutants responded characteristically to the compound, and all were as sensitive to it as the wild-type strain, then a potential new class of anti-nematode drug would have been uncovered.

In another preferred embodiment, bioactivity properties in compounds are identified by screening the compound against a library of mutant nematodes without first screening against the wild-type nematode. This collection may or may not include the wild-type nematode as one of the tests. For example, if the characteristic effect of the class of compounds is to alter the locomotive or egg-laying behavior of the a specific mutant, whereas this same compound has no known effect on wild-type animals, then new members of that class of compounds can be identified merely by exposing said mutant to the compound without first testing the compound against wild-type nematodes.

In a similar manner, if the characteristic response is the delay or induction of cell death, then new members of the class of compounds which possess the desired bioactivity may be identified utilizing cell death mutants without first testing the compound against wild-type nematodes.

It is not necessary that the compound being tested be in a purified form. The methods of the invention can be used to identify the presence of a desired bioactivity in an un-purified preparation. The methods of the invention may also be used to follow the purification of the desired bioactivity through various enrichment steps such as chromatography, electrophoresis, and other purification techniques known to those skilled in the art.

The present invention contemplates the use of a library of mutants which contains mutants displaying characteristic responses to many different classes of compounds. A parti-

cular utility of the invention is that different libraries can be assembled for different screening purposes.

Therefore, in a preferred embodiment, the library of mutants contains samples of stable nematode mutants representative of a wide range of pharmacological and chemical classes and biochemical targets. In another embodiment, the collection of mutants contains stable nematode mutants resistant to only one class of compound or to the activity of one gene's product or biochemical target.

The methods of the invention are also useful in the identification of different sub-classes of activities in a group of compounds otherwise believed to act through the same mechanism of action. For example, if compounds, all believed to be in the same pharmacological class, result in different activity profiles when screened against a library composed of mutants specific for that class, the results would be indicative of the existence of sub-classes of specific targets within the pharmacological class. Such a screen could be used to rapidly and clearly classify related bioactive agents into different subgroups of closely related bioactivities.

The invention envisions that a library containing wild-type and mutant nematodes, each of which responds in a different characteristic way, can be assayed collectively in any apparatus that collectively holds or separates the nematodes from each other. For example, microtiter dishes containing multiple compartments can be used, different mutants in different compartments, for both agar bed or liquid culture conditions.

Having now described the invention in general terms, the same will be further described by reference to certain specific examples that are provided herein for the purposes of explanation only and are not intended to be limiting unless otherwise specified. In examples 1-3, the characteristic response of the mutants is drug resistance. In example 4, the

characteristic response is drug sensitivity. In examples 5 and 6, the characteristic response is induced egg-laying.

Common characteristic response to compounds which have a common mode of action.

Example 1 -- Chemically similar compounds.

Benomyl, a benzimidazole carbamate, prevents the growth of wild-type nematodes. Mutant strain NS1004 was selected for resistance to benomyl. This example was designed to determine whether NS1004 also responds characteristically, i.e. displays resistance, to two other benzimidazole carbamates.

Method: Eggs from N2 (wild-type) or from NS1004 nematodes were placed on agar plates containing various concentrations of the compound to be tested. Control plates received no compound. The plates were maintained at 20°C and examined for growth of the nematode after 4 days. Plates containing adults and eggs and larvae of the next generation were scored '+'; if no growth (no adults or eggs and larvae of the next generation) had occurred the plate was scored '-'; if growth had occurred, but at a rate slower than that observed in the absence of drug, the plate was scored 'slo'.

Result: As shown in Table I, mutant strain NS1004 nematodes were able to grow and reproduce at concentrations of benomyl which prevented the growth of wild-type nematodes. NS1004 animals also grew at concentrations of the other benzimidazole carbamates (Compounds B and C) that prevented the growth of wild-type nematodes, i.e. NS1004 is cross-resistant to compounds B and C. This result suggests that benomyl, compound B and compound C all act by a common mode of action.

Example 2 -- Chemically different compounds.

This example was designed to determine whether strain NS1004 is also resistant to thiabendazole, a benzimidazole which lacks the carbamate group and is thought to have a mode of action which differs from the benzimidazole carbamates.

Methods: Same as example 1.

Result: As shown in Table I, mutant strain NS1004 responded characteristically to, that is, was resistant to the growth inhibitory properties of thiabendazole. This result suggests that the mode of action of thiabendazole is the same as the benzimidazole carbamates. The results further suggest that the carbamate group found in benomyl (compound A) and in compounds B and C, but not in thiabendazole (compound D), is not an essential group for the bioactive effect of this class of anti-nematode drugs; these results demonstrate the utility of nematode mutants for the rapid characterization of the essential bioactive elements of a complex organic compound. Further, these results demonstrate the use of the methods of the invention for the identification of a nematode mutant, NS1004, that may be used to identify other compounds which act by the same mechanism as benomyl.

TABLE I

	Compound							
	A		B		C		D	
Nematode Strain	N2	NS1004	N2	NS1004	N2	NS1004	N2	NS1004
Compound Dilution								
Undiluted	-	+	-	+	-	+	-	+
1/2	-	+	-	+	-	+	-	+
1/4	-	+	-	+	-	+	-	+
1/8	-	+	slo	+	-	+	-	+
1/16	-	+	+	+	-	+	slo	+
1/32	slo	+	+	+	-	+	+	+
1/64	+	+	+	+	+	+	+	+
1/128	+	+	+	+	+	+	+	+
Control	+	+	+	+	+	+	+	+

Compound A -- Benomyl, 1-[(Butylamino)carbonyl]-1H-benzimidazol-2-yl carbamic acid methyl ester (undiluted = 100 μg/ml);

Compound B -- Mebendazole, methyl 5-benzoyl-2-benzimidazole carbamic acid methyl ester (undiluted = 1 mg/ml);

Compound C -- Methyl [5-(2-thienylcarbonyl)-1H-benzimidazol-2-yl] carbamate (undiluted = 100 μg/ml);

Compound D -- Thiabendazole, 2-(4-thiazolyl)-1H-benzimidazole (undiluted = 1 mg/ml).

A library of mutant strains with characteristic responses to compounds which have different modes of action.

Example 3 -- Drug-resistant mutants.

Mutant strains NS1001, NS1002, NS1003, NS1004, and NS1005 form a library of strains which may be used to characterize the mode of action of putative new anti-nematode drugs. Each strain responds characteristically, i.e. displays resistance, to compounds in one of five different classes of anti-nematode drugs. To demonstrate the specificity of the characteristic response, each strain was tested for resistance to a compound in each of the five classes of drugs.

Methods: Eggs from wild-type (N2) and from each of the mutant strains were placed on agar plates containing various concentrations of the compound to be tested. After 4 and 10 days, the plates were examined for growth as described in Example 1. If the sensitivity of the mutant to the drug was the same as that of wild-type (N2) animals, the mutant was scored as being sensitive (S) to the compound. If the mutant was more resistant to the compound than wild-type animals, it was scored (R).

Results: As can be seen from Table II, each of the mutants displayed a characteristic response (i.e. resistance) to only one representative compound. In each case the compound to which the strain was resistant is in the same compound class as the drug which was used for the selection of the mutant strain. This suggests that each of the classes of anti-nematode drugs tested acts through a different target in the nematode. The data also demonstrate the use of the methods of the invention to eliminate potential modes of action as explanations of a compound's bioactivity. In particular, the

results in Table II clearly show that the mode of action of ivermectin (compound E), which is unknown, is not the same as that of the any of the other anti-nematode drugs tested.

Example 4 -- Drug-sensitive mutant.

Mutant strain NS1009 was selected, in a screen for behavioral mutants, for its distinctive severely uncoordinated locomotory behavior. Subsequent testing of various compounds revealed that treatment with α-endosulfan caused NS1009 animals to produce the smooth, coordinated locomotory behavior characteristic of wild-type nematodes. To demonstrate the specificity of this response we tested i) the effect of α-endosulfan on the wild-type nematodes and nematodes in the library of drug-resistant strains and ii) the effect of compounds representative of the five classes of anti-nematode drugs on strain NS1009.

Methods: Same as example 3.

Results: As can be seen in Table II, none of the strains in the library responded characteristically, in this case displayed sensitivity, to α-endosulfan. Also, NS1009 animals were not observed to be cross-resistant to any of the anti-nematode drugs.

This result illustrates the assertion that some mutant strains may respond characteristically to compounds which have no apparent effects on wild-type animals.

TABLE II

COMPOUNDS	A	B	C	D	E	F
Nematode Strain						
N2	S	S	S	S	S	R
NS1001	R	S	S	S	S	R
NS1002	S	R	S	S	S	R
NS1003	S	S	R	S	S	R
NS1004	S	S	S	R	S	R
NS1005	S	S	S	S	R	R
NS1009	S	S	S	S	S	S

Representative Compound

A -- aldicarb, representing acetylcholinesterase inhibitors;
B -- levamisole, representing acetylcholine receptor agonists;
C -- piperazine, representing GABA receptor agonists;
D -- thiabendazole, representing anti-tubulin drugs;
E -- ivermectin, representing avermectins (mode of action unknown);
F -- α-endosulfan, (mode of action unknown).

Egg-laying as a response to serotonin-related compounds.

Compounds which act as serotonin receptor agonists or serotonin uptake blockers cause wild-type egg-bearing hermaphrodites to lay eggs. Trent, C., supra. Compounds which are effective inducers of egg-laying were identified by screening for their ability to induce egg-laying in wild-type nematodes. The collection of compounds screened included compounds known to be serotonin receptor agonists and serotonin uptake blockers in mammals.

Example 5

Mutant strains NS1006 and NS1007 were selected in screens for animals which were defective in egg-laying. Trent, C., supra. The response of these mutants to serotonin receptor agonists and serotonin uptake blockers which stimulate egg-laying in the wild-type nematode was examined.

Methods: Single egg-bearing adult hermaphrodites were placed in the well of a 96 well plate with 100μl of M9 buffer (Brenner, supra) containing the compound to be tested. After 90 minutes, at 20^{o}C, the number of eggs which had been released were counted. If the compound induced the release of an average of 10 or more eggs per animal it was scored as '+'. A weak response, defined as the release of less than 5 eggs released per animal is indicated by 'weak'. Controls containing no, or inactive compounds, generally released an average of less than one egg per animal in the same period; inactive compounds were scored as '-'.

Results: As can be seen from Table III, animals from mutant strain NS1006 laid eggs (behaved like wild type nematodes) when exposed to serotonin agonists. In contrast,

they responded characteristically (did not lay eggs) when exposed to serotonin uptake blockers. NS1007 animals responded characteristically (did not lay eggs), or laid less than 5 eggs per animal, when exposed to serotonin agonists, but did lay eggs in response to some serotonin uptake blockers (herein called subclass A blockers). Other serotonin uptake blockers (herein called subclass B blockers) induced no release of eggs from NS1007 animals.

These results show that the characteristic lack of egg-laying responses of NS1006 and NS1007 to compounds which induce egg-laying in wild-type animals can be used to distinguish serotonin agonists from serotonin uptake blockers. In addition mutant strain NS1007 showed a differential response to serotonin uptake blockers allowing the separation of these compounds into two subclasses. No other methods for the separation of these subclasses are known.

TABLE III

Compound Name	N2	NS1006	NS1007
A. Serotonin agonists			
5-hydroxytryptamine (serotonin)	+	+	-
2-methyl-5-hydroxytryptamine	+	+	-
quipazine	+	+	-
1-(2,5-dimethoxy-4-indophenyl)-2-aminopropane	+	+	weak
B. Subclass A Serotonin Uptake Blockers			
clomipramine	+	-	+
imipramine	+	-	+
doxepin	+	-	+
amitriptyline	+	-	+
nortriptyline	+	-	+
viqualine	+	-	+
C. Subclass B Serotonin Uptake Blockers			
fluoxetine	+	-	-
paroxetine	+	-	-
sertraline	+	-	-

Example 6

Characteristic Responses related to Cell Death

Achieving therapeutic control over cell death is an important goal of current drug discovery research. Cell death is divided, based on morphological criteria into two types: apoptotic cell deaths and necrotic cell deaths. Apoptosis plays an important role in biological development. For example, development of the vertebtrate hand or wing requires that carefully designated populations of cells die at precisely determined developmental stages. Apoptotic cell death is also important during insect and amphibian metamorphosis. Apoptosis is characteristic of normal tissues that have cell turnover, for example, as found in various

lymphoid tissues such as the thymus, lymph nodes and spleen. Apoptosis has also been implicated in clonal deletion during T-cell response to normal hormonal changes. Low levels of noxious agents such as cytotoxic drugs and radiation increase apoptosis. Apoptosis is also a feature of tumor development. Induction of apoptosis can, in some instances, cause tumor regression.

Necrotic cell deaths are observed most often in disease states.

The pathophysiology of disease can involve either apoptotic or necrotic cell death (Walker, N.I. et al., Methods Achiev. Exp. Pathol. 13:18-54 (1988)). For this reason, blockers of apoptosis or necrotic cell death might be useful in preventing degenerative diseases. Alternatively, agonists of cell death might be used to destroy neoplastic growth or, properly controlled, as pest control agents.

As in most animal species, apoptotic cell death is a normal part of C. elegan development (Sulston, J.E. and Horvitz, H.R., Develop. Biol. 56:110-156 (1977); "The Nematode Caenorhabditis elegans," W.B. Wood, ed., 1988, Cold Spring Harbor Laboratory, especially Chapters 5 and 6). In hermaphrodites, of the total 1090 nuclei generated by embryonic lineages, 131 undergo programmed apoptotic deaths. These deaths can be observed in living animals with differential interference contrast microscopy.

Several aspects of C. elegans programmed cell death have been subjected to genetic analysis. In some mutant strains the engulfment and/or degradation (i.e., phagocytosis) of dead cells is blocked. In these strains readily visible remnants of dead cells can be observed for extended periods of time, sometimes into adulthood. The presence of remnants is a characteristic anatomical feature of programmed cell death. These features would be absent if these mutant animals were

grown in the presence of compounds which abolished apoptotic cell death, i.e., in which no programmed cell deaths occur.

Cell death may also produce a behavior effect which can be utilized to identify compounds with a desired activity. For example, the mutation in C. elegans strain NS1011 causes cells which normally form the vulva to die prematurely. As a result, no vulva is formed. The characteristic behavior of NS1011 animals is that they cannot lay eggs. It is known that strains which carry both the mutation present is NS1011, and in addition, a second mutation which blocks phagocytosis in the vulva precursors, survive and the animals display normal egg-laying behavior. Consequently, it can be expected that antagonists of phagocytosis would cause the development of egg-laying competent animals from strain NS1011.

In other mutant strains, nerve cells which normally survive, and perform functions necessary for normal behavior, instead undergo apoptosis. The latter strains display characteristic behavioral abnormalities which are dependent upon the occurrence of cell death. The mutation present in the strain NS1006, for example, causes the (inappropriate) apoptosis of the motor neurons which innervate the vulva muscles. In the absence of these neurons, NS1006 animals are unable to lay eggs. It is known that in strains carrying both the mutation present in NS1006 and, in addition, a second mutation which blocks apoptosis, the vulva motor neurons survive and the animals display normal egg-laying motor behavior. Consequently, we expect that antagonists of apoptosis would cause the development of egg-laying competent animals from strain NS1006.

A number of mutant strains of C. elegans have been identified in which specific cells undergo necrotic deaths. The mutation carried in the strain NS1010, for example, causes specific cell deaths. Compounds which block specific

necrotic cell death would cause the development of touch responsive animals from strain NS1010.

Having now fully described this invention, it will be understood by those with skill in the art that the same may be performed within a wide and equivalent range of conditions, parameters, and the like, without affecting the spirit or scope of the invention or of any embodiment thereof.

WHAT IS CLAIMED IS:

1. A method of screening and classifying a bioactive compound whose mode of action is unknown, comprising:

evaluating the effect of said bioactive compound on the characteristic response of at least one mutant nematode; and

comparing the activity profile of said bioactive compound with that of a compound whose activity profile is known; and

classifying said compound based upon its activity profile.

2. The method of claim 1, wherein said mutant nematode responds characteristically to a synthetic compound.

3. The method of claim 1, wherein said mutant nematode responds characteristically to a natural product.

4. The method of claim 1, wherein said mutant responds characteristically to a compound selected from the group consisting of a pharmaceutical, anti-nematode compound, pesticide, insecticide, fungicide, parasiticide, and endectoside.

5. The method of claim 4, wherein said anti-nematode compound is selected from the group consisting of an anthelmintic and nematocide.

6. The method of claim 1, wherein said mutant nematode responds characteristically to a neuroactive compound.

7. The method of claim 6, wherein said neuroactive compound is selected from the group consisting of an acetylcholinester-

ase inhibitor, acetylcholine agonist, acetylcholine antagonist, GABA agonist, GABA antagonist, dopamine agonist, dopamine antagonist, antitubulin agent, serotonin agonist, serotonin antagonist and serotonin uptake blocker.

8. The method of claim 7, wherein said neuroactive compound is an acetylcholinesterase inhibitor.

9. The method of claim 7, wherein said neuroactive compound is an antitubulin agent.

10. The method of claim 7, wherein said neuroactive compound is a serotonin uptake blocker.

11. The method of claim 10, wherein said serotonin uptake blocker is a subclass A blocker.

12. The method of claim 10, wherein said serotonin uptake blocker is a subclass B blocker.

13. The method of claim 1, wherein at least one characteristic response involves the locomotion and coordination of a nematode.

14. The method of claim 1, wherein at least one characteristic response involves the egg-laying rate of a nematode.

15. The method of claim 1, wherein at least one characteristic response involves the feeding activity of a nematode.

16. The method of claim 1, wherein at least one characteristic response involves a change in the taxis behavior of a nematode.

17. The method of claim 1, wherein said compound results in the death, paralysis, lack of growth, or infertility of a wild-type nematode.

18. The method of claim 1, wherein said compound inhibits or prevents cell death.

19. The method of claim 1, wherein said compound accelerates or induces cell death.

20. The method of any one of claims 18 or 19, wherein said cell death is the result of apoptosis.

21. The method of any one of claims 18 or 19, wherein said cell death is the result of necrosis.

22. A method of screening and classifying a compound comprising:

evaluating the effect of said compound on a wild-type nematode;

evaluating the effect of said compound on the characteristic response of at least one mutant nematode;

comparing the activity profile of said compound with that of a compound whose activity profile is known; and

classifying said compound based upon its activity profile.

23. The method of claim 22, wherein said mutant nematode responds characteristically to a synthetic compound.

24. The method of claim 22, wherein said mutant nematode responds characteristically to a natural product.

25. The method of claim 22, wherein said mutant responds characteristically to a compound selected from the group consisting of a pharmaceutical, anti-nematode compound, pesticide, insecticide, fungicide, parasiticide, and endectoside.

26. The method of claim 25, wherein said anti-nematode compound is selected from the group consisting of an anthelmintic and nematocide.

27. The method of claim 22, wherein said mutant nematode responds characteristically to a neuroactive compound.

28. The method of claim 27, wherein said neuroactive compound is selected from the group consisting of an acetylcholinesterase inhibitor, acetylcholine agonist, acetylcholine antagonist, GABA agonist, GABA antagonist, dopamine agonist, dopamine antagonist, antitubulin agent, serotonin agonist, serotonin antagonist and serotonin uptake blocker.

29. The method of claim 28, wherein said neuroactive compound is an acetylcholinesterase inhibitor.

30. The method of claim 28, wherein said neuroactive compound is an antitubulin agent.

31. The method of claim 28, wherein said neuroactive compound is a serotonin uptake blocker.

32. The method of claim 31, wherein said serotonin uptake blocker is a subclass A blocker.

33. The method of claim 31, wherein said serotonin uptake blocker is a subclass B blocker.

34. The method of claim 22, wherein at least one characteristic response involves the locomotion and coordination of a nematode.

35. The method of claim 22, wherein at least one characteristic response involves the egg-laying rate of a nematode.

36. The method of claim 22, wherein at least one characteristic response involves the feeding activity of a nematode.

37. The method of claim 22, wherein at least one characteristic response involves a change in the taxis behavior of a nematode.

38. The method of claim 22, wherein said compound results in the death, paralysis, lack of growth, or infertility of a wild-type nematode.

39. The method of claim 22, wherein said compound inhibits or prevents cell death.

40. The method of claim 22, wherein said compound accelerates or induces cell death.

41. The method of any one of claims 39 or 40, wherein said cell death is the result of apoptosis.

42. The method of any one of claims 39 or 40, wherein said cell death is the result of necrosis.